THE IDENTIFICATION OF THE PRECURSOR OF OFF ODOR CAUSING COMPOUNDS, PRODUCED DURING STORAGE OF GROUND PEARL MILLET (*Pennisetum americanum* [L] Leeke)

by

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Major professor

This thesis is dedicated to my parents.

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INTRODUCTION

Pearl millet (Pennisetum americanum L. Leeke) is grown in the largest amounts and has the greatest potential, of the millets. It has good yielding potential of both grain and fodder, and is considered more efficient in its utilization of moisture and has a higher tolerance of heat than does sorghum or maize. Pearl millet is grown extensively as a food crop in the drier areas of West and South India and along the Southern peripheries of the Sahara desert in Africa. It is cultivated as a forage crop in the Southeastern United States, Australia, and South Africa (Mujumdar, 1980).

The average annual world production of millets was estimated at about 44 million tons of grain as an average for the five year period 1967-71 (Mujumdar, 1980). Approximately 85% of this production was utilized for human food, and was sufficient to feed 400 million people. Pearl millet is the principal staple of the diets in parts of India and some countries in Africa, where cereals constitute 80 to 90% of the total caloric intake.

Traditionally, millets have been considered "poor man's bread" and legumes as "poor man's meat". In general, PER (protein efficiency ratio) of pearl millet is higher than for wheat or sorghum (Oke, 1977, Pushpamma et al, 1972, Rao et al, 1964). Based on caloric requirement, studies show that millet satisfies the requirement for protein, iron, and vitamins B_1 and B_2. Pearl millet does not contain any tannis (Butler, L., unpublished data). The level of phytic acid in pearl millet is slightly lower than that reported for wheat and appears to vary both with location and with cultivar (Simwemba et al., 1984).

Because of its small seed size and hard endosperm pearl millet is difficult to mill. In developing countries, mortar-and-pestle grinding, and stone grinding methods are still common in the rural areas. Using such techniques, one Senegalese must spend up to six hours to mill sufficient millet for use in preparing one days food for a family (Varriano-Marston and Hoseney, 1983).

Pearl millet can be stored for long periods without significant changes in quality if the kernels remain intact, but once the grain is subjected to grinding the meal quality rapidly deteriorates (Varriano-Marston & Hoseney, 1983). The meal develops a mousy acidic odor within a few hours after grinding. The objectives of this work are to identify the off odor causing compound(s) in processed pearl millet.

LITERATURE REVIEW

Kernels of most pearl millet cultivars are tear shaped and weigh about 9 mg or a third of the weight of sorghum kernels (Hoseney et al, 1981). A scanning electron micrograph of the fractured kernel shows that germ is large in proportion to the rest of the kernel. Germ was found to be 17% of the medium size kernel (Abdelrahman et al, 1984).

A number of traditional and mechanized processes have been used to mill pearl millet. Extraction rates using those processes range from about 75% to about 85% (Lai & Varriano-Marston, 1980b). Machines for dehulling of paddy rice and milling wheat are available in developing countries. However, machines for dehulling coarse grains are not yet available. Generally millet is processed at home on a daily basis (fig. 1). Decortication is accomplished by pounding the moistened grain, air drying it, and removing the bran and fine material by winnowing. Village stone mills are then used to grind the coarse particles into flour (Varriano-Marston and Hoseney, 1983).

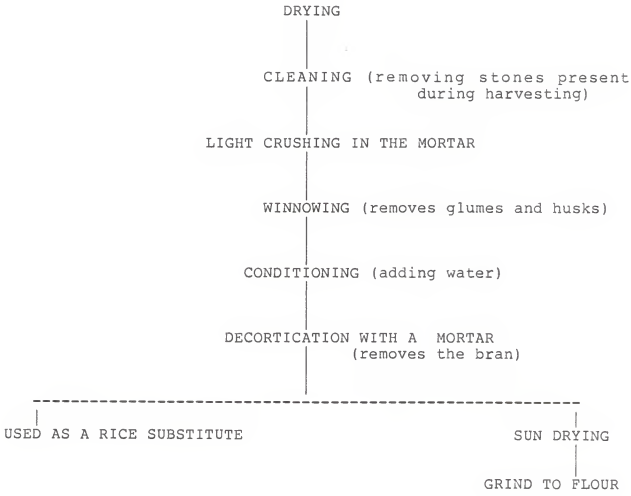
LIPIDS

Lipid components are generally considered to be responsible for the deterioration of ground pearl millet. The milling processes do not completely separate the germ from the rest of the kernel. Therefore, it is generally thought that development of milling processes that effectively remove the major lipid containing portions of the grain i.e. the germ and covering layers should produce stable ground products (Varriano-Marston and Hoseney, 1983).

The level of free lipids (petroleum ether extractable) extracted from pearl millet cultivars varies from 3.03% to 7.4% (Lai and

Fig. 1

TRADITIONAL PROCESSING OF PEARL MILLET.


DRYING
CLEANING (removing stones present during harvesting)
LIGHT CRUSHING IN THE MORTAR
WINNOWING (removes glumes and husks)
CONDITIONING (adding water)
DECORTICATION WITH A MORTAR (removes the bran)
USED AS A RICE SUBSTITUTE
SUN DRYING
GRIND TO FLOUR

Varriano-Marston, 1980a, Rooney, 1978). The principal fatty acids in free lipids are oleic, linoleic, palmitic, stearic and myristic (Agarwal and Sinha, 1964). Bound lipid (not extractable with petroleum ether) contents ranged from 0.58 to 0.90% among 18 samples of pearl millet grown in Kansas (Lai & Varriano-Marston, 1980a).

Lai & Varriano-Marston (1980a), tentatively identified the nonpolar components of both free and bound millet lipids as, hydrocarbons, and steryl esters, triacylglycerols, free fatty acids and partially acylated glycerols. Lai & Varriano-Marston (1980a) concluded that qualitatively, the fatty acids found in free lipids except C22:0 were found also in bound lipids. However, C18:2 was present in lower weight percentage in bound than in free lipids. In addition, they also reported five other fatty acids in bound lipids C10:0, C12:0, C13:0, C15:0 and C24:0. The total percentage of long chain fatty acids was greater in bound lipids than in free lipids. Freeman and Bocan (1973) reported that pearl millet oil tends to be higher in palmitic and stearic acid and lower in oleic acid than oil from either grain sorghum or corn. The level of linoleic acid was found to be similar to that of sorghum but higher than that of corn oil.

STORAGE STABILITY

Duese and Perton (1970) stated that millet is prone to "rancidness" during storage owing to the high level of lipids (up to 5%), as compared to wheat (2%). The important problem of aging of food has been related to various forms of biological oxidation of lipids (Frankel, 1982). The biochemical processes that occur during storage of grain products lead to both hydrolytic and oxidative decomposition of lipids.

Hydroperoxides (ROOH) are the primary products of enzymatic and nonenzymatic lipid oxidation. Although fat hydroperoxides are tasteless and odorless, their decomposition products have a great impact on flavor. They often impart an objectionable odor and taste to food products. Some volatile cleavage products are extremely potent and can effect the flavor of vegetable oils at concentrations as low as 1 ppm (Frankel, 1982). Such unsaturated aldehydes and ketones, formed as primary decomposition products are obvious sources of additional volatile products due to their susceptibility to further oxidation (Frankel, 1982). The complicated set of reaction pathways is capable of producing a multitude of volatile and nonvolatile products.

Alkoxy radicals are precursors of most stable oxidation products (Frankel, 1982). Bell et al, (1981) proposed that alkoxy radicals may be formed by three routes:

Homolytic cleavage of hydroperoxides:

$$ROOH \text{----------} RO\cdot + \cdot OH \qquad I$$

Interaction of peroxy radicles:

$$ROOR \text{----------} 2RO\cdot + O_2 \qquad II$$

Homolytic cleavage of a peroxide:

$$ROOR \text{----------} 2RO\cdot \qquad III$$

Secondary alkoxy radicals may cleave to form aldehydes:

$$R_2' CHO\cdot \text{----------} R'CHO\cdot + R'. \qquad IV$$

Alkoxy radicals may also react with substrate:

$R_2'CHO\cdot$ ---------- $R_2'CHOH + R\cdot$ V

Reactions IV and V generate alkyl radicals and, therefore, propagate the reaction. The alkoxy radicals may also react with the above mentioned alkyl radicals:

$R_2'CHO\cdot$ ---------- $R_2'CO\cdot + RH$ VI

or with another alkoxy radical:

$R_2'CHO$ ---------- $R_2'CO\cdot + ROH$ VII

The last two reactions terminate the reaction by forming stable products.

Most of our knowledge of the volatile products resulting from lipid oxidation is based on the identification of the stable volatile products that are most amendable to currently used separation and analytical techniques (Frankel, 1982). Most research on odor generation also assumes that the volatile oxidation products are derived mainly from the breakdown of monohydroperoxides. Frankel (1982) suggests that secondary nonvolatile products of hydroperoxides can also decompose and contribute significant amounts of volatile oxidation products.

EFFECT OF STORAGE ON MILLET LIPID

Storing millet flour at 30° C and 95% relative humidity for three months produced a 3% decrease in ether extractable lipids according to (Carnovale and Quagalia, 1973). No explanation was given for the decrease. Nachaev et al (1973) showed essentially no change in total ether-extractable lipids in millet during storage. Carnovale and Capital (1973) used infra-red to study extracted lipids, and showed that

the increases in absorption relate to the increases in free carboxylic acid group. These findings indicate an increase in fat acidity and suggest that hydrolytic as well as oxidative changes in lipid occur during storage of millet flour. Lower grade flours changed more rapidly and more markedly than refined flours. Given the relative amount of lipids in each, this could be anticipated. Thiam et al (1976) suggested that the major cause of quality deterioration in millet flours during storage was microbial growth. However, they found that millet flour (85% extraction) stored at 30° C and 50% RH for four months showed neither odor nor taste deterioration. Conversely, odor changes were detected by Lai and Varriano-Marston (1980b) in millet meal stored for only 4.5 days at 19° C and 58% RH. They found that time at which odor changes were detectable corresponds to fat acidity of 30 mg KOH required to titrate free fatty acids in 100 g of meal. They also observed a reduction in mold count during the first six weeks of storage. This questioned the concept that fungal growth may have any effect on odor changes observed in the first week of storage (Lai and Varriano-Marston, 1980b).

Lai & Varriano-Marston (1980b) reported that a taste panel noticed that temperatures and humidities shortened the time necessary before "aged" characteristics were detected in ground millet. Their olfactory data showed significant changes in odor perception by the panelists for samples stored for 12 hours at 42° C and 75% RH. On the other hand, 60 and 108 hours, respectively, were required before significant odor changes were noted for millet meals stored at 27° C, 64% RH and 19° C, 58% RH.

Kaced et al (1984) found that when millet meal was stored in polyethylene bags, it's peroxide value increased rapidly and appeared to

signal the start of rancidity. However, millet meal stored in cotton bags showed no peroxide accumulation. This may suggest that the procedure measures unknown volatiles and not peroxides. Kaced et al (1984) concluded that free fatty acids and fat acidity data showed that all acidity produced during storage was the result of free fatty acids. The authors also reported that under high relative humidity (90%), fat acidity increased considerably during the first few days of storage and then levelled off.

Hexanal is a major product of oxidative degradation of lipids (Fristch and Gale, 1977). Kaced et al (1984) found no hexanal in millet meal stored for 15 days in polyethylene bags. They found that, as in other cereals, hexanal concentration increases after 21 days of storage but, that did not correlate with the rancid odors and flavors produced earlier during storage of millet meal.

PHENOLIC ACIDS

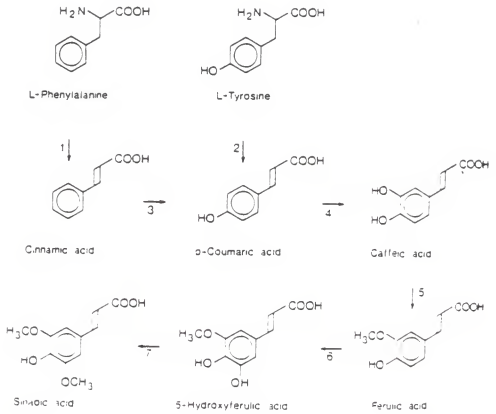
Phenolic acids are one of the most important classes of secondary plant products. They are very reactive and easily undergo oxidation, substitution, and coupling reactions (Conn, 1981). The term "phenolic acids" is applicable to a large variety of different organic compounds bearing at least one phenolic hydroxyl group and a carboxylic function. It is common practice, however, to use this designation in connection with only a limited number of natural products, namely cinnamic acids (phenyl acrylic acids) and benzoic acids (phenyl carboxlic acids) (Conn, 1981).

It is well documented that the carbon skeleton of cinnamic acids originates from aromatic amino acid L-phenylalanine (fig. 2). Cinnamic acid undergoes ring substitution in a series of hydroxylation and

Fig. 2

THE PNENYL ALANINE-CINNAMATE PATHWAY: 1)phenyl alanine ammonia-lyase (2)tyrosine ammonia-lyase (3)cinnamate 4-hydroxylase (4) p-coumarate 3-hydroxylase (5 & 7)catechol o-methyl transferase (6)ferulate 5-hydroxylase(hypothetical).

[Reproduced from: The Biochemistry of Plants. Ed. E.E.Conn. Academic Press, New York]


L-Phenylalanine
L-Tyrosine
Cinnamic acid
p-Coumaric acid
Caffeic acid
Sinapic acid
5-Hydroxyferulic acid
Ferulic acid

methylation steps yielding various p-hydroxylated cinnamic acids (Conn, 1981). The most important mechanism for the formation of benzoic acids in plants is the side chain degradation of cinnamic acids. The three benzoic acids, p-hydroxybenzoic, vanillic and syringic are constitutents of lignin (Harborne and Simmonds, 1964).

There is an increasing interest in the effect of phenolic acids on food quality. A few phenolic acids which occur in relatively high concentrations in oil seeds and legume seeds have been implicated in the development of adverse tastes and colors in food products or decreasing the protein nutritive value (Sosulski, 1982). Maga and Lorenz (1973) have shown that phenolic acids can contribute objectionable flavors, especially astringency, at taste threshold levels of 40 to 90 ppm (Sosulski, 1982). Several phenolic acids and their derivatives are known to contribute grey, brown or green colors to food products (McClure, 1975). Sinapine a choline ester of sinapic acid occurs in rape seed meals at levels of 1.0 to 2.5% (under Canadian conditions). In addition to its adverse effects on meal flavor and palatability, it was found to be responsible for the fishy odors in brown-shelled eggs produced by hens fed ration containing sinapine (Hobson-Frohock et al, 1975).

Milling and cooking processes influence pearl millet PER and digestibility (Hoseney et al, 1981). Dassenko (1980) showed that PER increased significantly when pearl millet was milled to a 67% extraction. Milling the grain to flour improved the PER, suggesting that a component(s) in the outer layers of the grain decreased the PER. The color of pearl millet varies from off-white to dark brown. The most common and generally preferred color is slate grey. In parts of Africa, millet is often soaked in water containing tamarind pods or sour milk

(Vogel and Graham, 1979). This soaking alters the flavor and bleaches the grain.

Reichert (1979) found pH sensitive pigments in pearl millet which appeared to be responsible for the natural grey color of the outer layers of its endosperm. These were the flavonoids; gluscosylvitexin, glucosyloreintin, and vitexin. Refluxing the millet flour with methanol following petroleum ether pre-extraction effectively extracted these C-glycosylflavones. Reichert's (1979) data showed pearl millet contained 124 mg of C-glcosylflavones in 100 gms of grain. This amount decreases markedly but not completely upon dehulling.

FLAVONOIDS

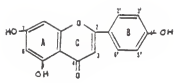
Flavonoids are polyphenols which are found in virtually all parts of the plant, the root, heartwood, sapwood, bark, leaf, fruit and flower (Harborne et al, 1975). The chemical structure of flavonoids is based on a 15 carbon skeleton with a chromane ring bearing a second aromatic ring B in position 2 and 3 (fig. 3). The carbon skeleton of all flavonoids is derived from acetate and phenylalanine. Ring A is formed of 3 acetate units, while phenylalanie gives rise to Ring B and C-2, C-3 and C-4 of the heterocyclic Ring C.

There are six major groups of flavonoids, chalcones, flavanones, flavones, flavanols, isoflavanes and anthocyanidins (Swain et al, 1979). Chromatographic analyses have shown the frequent occurrence of compounds which present the solubility and chromatographic properties of flavonoid glycosides, but which unlike flavonoid glycosides cannot be hydrolyzed even after prolonged treatment with acid. This group of flavonoids are called C-glycosyl flavonoids and their resistance to acid hydrolysis

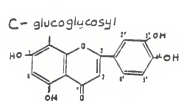
Fig. 3

C-GLYCOSYLFLAVONES. A, Apigenin (4',5,7, trihydroxy flavone); B, glucosyl orientin; C, vitexin; D, glucosyl vitexin.

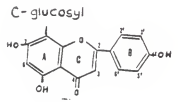
A



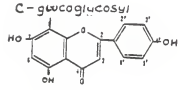
B



C



D



results from the sugar being directly linked to the flavonoid nucleus by a carbon-carbon bond (Harborne et al, 1975).

C-glucosylflavonoids are rather widely distributed in the plant kingdom. They have now been found in dicotyledons, monocotyledons, ferns, mosses and green algae (Harborne et al, 1975). Any part of the plant may be a source of C-glycosylflavonoids, but they are frequently found in the aerial parts. Vitexin, (8-C-B-D-glucopyranosyl apigenin), the best known C-glycosylflavonoid, was first isolated from the wood of Vitex lucens (verbenaceae) by Perkins (1898) and was considered to be an unusual type of apigenin glucoside. The complete structure of vitexin was elucidated by Horowitz and Gentili (1964). Iso-Vitexin, (6-C-B-D-glucopyranosyl apigenin), was found to co-occur with vitexin in extracts of Vitex lucens wood by Perkin in 1898 hydrolysis products of soponarin by Berger in 1906 (Harborne et al, 1975). When heated in acid solution isovitexin and vitexin yield an equilibrium mixture of both, which is easily resolved by paper chromatography (Seikel and Geissman, 1957). Orientin, (8-C-B-D-glucopyranosyl iuteolin), was first isolated from polyonum orientale by Horohammer et al (1958) (Harborne et al, 1975).

The method of isolation of flavonoids depends to some extent on the source of material, and type of flavonoid being isolated. Solvents used for extraction are chosen according to the polarity of flavonoid being studied (Harborne et al, 1975).

The less polar solvents are particularly useful for the extraction of flavonoid aglycones, while the more polar solvents are used if flavonoid glucosides or anthocyanins are sought. Flavonoid glucosides

are generally isolated from plant material by extraction with acetone, alcohol, water or combination of these (Harborne et al, 1975).

Mabry et al (1970), isolated flavonoids from the leaves of Hymenoxys scaposa using successive extraction with cold petroleum ether, methylene chloride, methanol and aqueous methanol. Two dimensional paper chronmatographic analysis of those extracts showed that petroleum ether had not extracted any flavonoids, methylene chloride removed only a few flavonoid aglycones, while the methanol and aqueous methanol were rich in flavonoid glycosides and contained aglycones. Petroleum ether pre-extraction removes a number of non-flavonoid constitutents such as fats and chloropryll and in some instances, was an essential step in order to obtain a workable extract of flavonoids with aqueous methanol.

Most phenolic compounds occur in plant leaf, stem or flower in water soluble forms, as glycosides. And every plant that has been examined has yielded a water soluble fraction in which the bulk of the low molecular weight phenolic material is present (Swain et al, 1979). In case of flavones, linkage of sugar to the carbon skeleton as in C-glycosylflavones is the form of conjugation which gives them the water soluble property (Swain et al, 1979). The possible toxicity of free phenolic acids may account for the fact that these compounds are nearly always present in the living tissues as glycosides (Swain et al, 1979). Conjugation with sugar therefore, has dual role in deactivating and in providing water solubility.

Glycosylation of phenolic hydroxyl groups has a functional significance in case of those flavonoids which are colored and occur in flower tissues. Variations in the position of attachment of the sugar to the flavonoid nucleus can lead to significant shifts in visible color

(Swain et al, 1979). According to Harborne (1977) glycosylation may be important in ecology of flavonoids in plants.

On the basis of histological examinations and coincidence of occurrence within the same tissue, Van Fleet (1969) suggested that a major function of flavonoids is to serve as antioxidants for lipids and polyacetylenes in plant tissues. Other important functions attributed to flavonoids are, protective agents, against UV light (Hohlbrock, 1981) or infections by phytopathogenic organisms (Bell, 1981). In polyphenols like flavonoids, the important active site is the catechol nucleus, which as the ability to chelate metals, and which may be directly involved in a number of biological reactions (Swain et al, 1979).

In case of flavonoids of the leaf, there are experiments which suggest that the nature of the sugar present may determine whether a particular flavonoid is an effective feeding deterrent to insects and grazing animals. Whether the flavonoid can be detected by taste is not known, and it is unfortunate that so few flavonoids have been extensively tested for taste properties (Horwitz, 1964). However, it is known in flavonone series that the structural requirement for bitterness is the presence of flavonone nucleus together with a particular disaccaride (neosheridose) attached to a particular phenolic hydroxyl (Horwitz, 1964).

Although the presence of C-glucosylflavones has been reported by Reichert (1979) in pearl millet, their contribution to palatability, storage and nutritional properties of the grain is not known.

MATERIALS AND METHODS

A blend of pearl millet varieties grown in Hays, Kansas and Garden City, Kansas were used for the study. Millet samples from three crop years 1980, 1981, 1982 were utilized.

White sorghum samples for the interchange studies were grown at Kansas Agricultural Experimental Station, Manhattan, Kansas.

Udy Cyclotech Laboratory Mill (Udy Corporation, Denver, CO) was used to mill the millet into a meal. Ross Laboratory Roller Mill was used to grind the Millet into grits.

ODOR PRODUCTION

MILLING THE MILLET SAMPLES. Initially the samples were ground with the Udy Cycglotech Laboratory mill into a meal. The effects of crop year (length of time the grain was stored), and moisture on odor generation was tested using this meal. However, a quicker method of odor production, and also higher intensity of odor was found when millet grits were used instead of meal. Millet grits were obtained by grinding with a Ross Laboratory Roller Mill with corrugated first break rolls. The roll diameter was 6" and the rolls were operated at a differential of 2.5:1. Mason jars with air tight seals were used for storage.

EFFECT OF STORAGE TIME. Three cleaned (removing husks and glumes), millet samples (20 g) from each crop year (1980, 1981, 1982) were ground with the Udy Mill, Moisture contents of the resulting millet meals were determined and each transferred to an air tight jar. Storage time was 20 days. The samples were evaluated by sniffing at regular intervals to determine the onset of odor generation.

EFFECT OF MOISTURE LEVEL. Pearl millet grain (crop year 1982) containing 14% moisture was tempered to 15.7%, 16.3% and 18.5% moisture by addition

of appropriate amounts of water. The moisture was allowed to equilibrate by storage for two hours at room temperature. After being ground to a meal with the Udy Mill, each sample was held in air tight jars for ten days. Sensory evaluation was performed daily to detect the onset of odor production.

EFFECTS OF SEQUENTIAL WETTING AND DRYING. Cleaned pearl millet grain (Hays, KS) was ground into grits by passage through a pair of corrugated rolls with a roll gap of 0.13". The grits were transferred to a flat glass or stainless steel tray. They were then mixed with 30% (W/V) water and immediately air dried at room temperature (60 to 70 F) in a hood or with a table fan. Drying time was 12 to 15 hours, after which time the moisture content of the grits closely approximated that of the product before wetting. Treated and dried samples were sealed in mason jars for at least one hour before being evaluated. Evaluation of odor generation was done by a panel of 3 to 5 members.

EFFECT ON TASTE. Millet meal was obtained by grinding 100 gms of either (a) wet then dried grits or (b) untreated grits (control). Gruels were prepared from each flour by mixing with 325 ml of boiling water (with stirring) and cooking for 30 minutes. After the gruel was cooled to room temperature a five member, untrained, panel was asked to judge the taste of the "wetted and dried" gruel as being the same or different from that of the control gruel (made from untreated grits).

FRACTIONATION AND RECONSTITUTION

EFFECT OF SOLVENT EXTRACTION. Millet grits were extracted with one of the four solvents, petroleum ether, chloroform, methanol and n-butanol (all reagent grade) as follows: Grits (500 gms) were placed in a large soxhlet and extracted for 12 hours with 2000 ml of solvent. Extracted

grits were air dried to remove the residual solvent before being wetted and dried (previously described) to test for odor production.

FRACTIONATION OF THE METHANOL EXTRACT. Pearl millet grits were defatted with petroleum ether extraction in a soxhlet for 16 hours. The defatted grits were air dried to remove residual solvent. The defatted grits (100 gms) were extracted with 300 ml of methanol for 12 hours in a soxhlet apparatus. Extracted grits were saved for future testing and extracting solvent was removed by rotary evaporation. The residue remaining after methanol removal was dissolved in 100 ml of petroleum ether. Petroleum ether was subsequently removed by rotary evaporation to produce the final extract.

Distilled water (300 ml) was added to the desolventized residue, and the mixture was stirred, allowed to stand for five minutes, and then filtered through a buchner funnel. The soluble material was considered "water solubles". The water insoluble material was recovered from the buchner funnel dried and resuspended in 300 ml of methanol, and solubles termed "methanol solubles".

RECONSTITUTION PROCEDURES. Methanol solubles and water solubles described above were reconstituted with the methanol extracted grits and tested as shown in figure 4.

INTERCHANGE STUDIES. Methanol extracts of sorghum grits and millet grits were obtained as previously described (fractionation-reconstitution). The extracts were then reconstituted and/or recombined as shown in figures 5 & 6.

Fig. 4

FRACTIONATION OF THE METHANOL EXTRACT FROM PEARL MILLET.

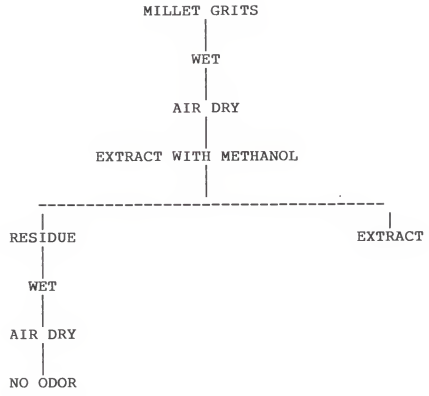

MILLET GRITS
WET
AIR DRY
EXTRACT WITH METHANOL
RESIDUE
EXTRACT
WET
AIR DRY
NO ODOR

Fig. 5

RECONSTITUTION OF MILLET GRITS WITH METHANOL EXTRACTS FROM SORGHUM.

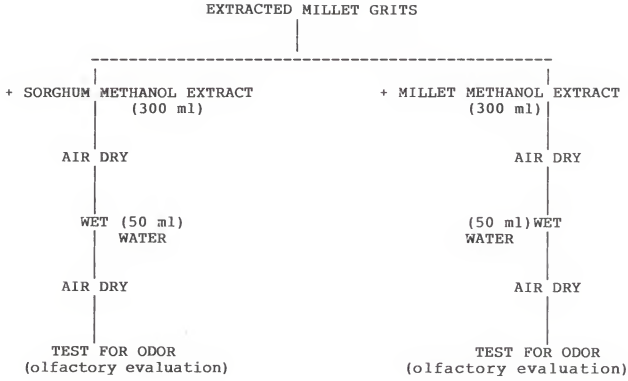
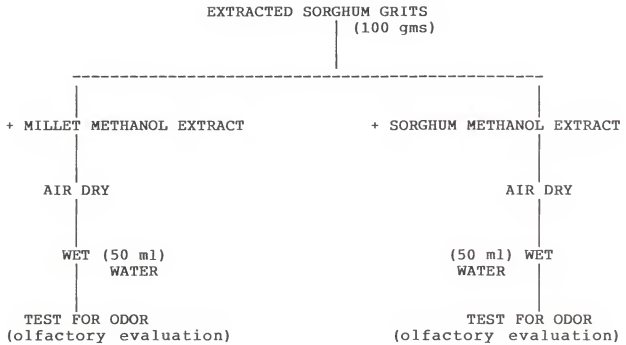

EXTRACTED MILLET GRITS
+ SORGHUM METHANOL EXTRACT (300 ml)
AIR DRY
WET (50 ml) WATER
AIR DRY
TEST FOR ODOR (olfactory evaluation)
+ MILLET METHANOL EXTRACT (300 ml)
AIR DRY
(50 ml) WET WATER
AIR DRY
TEST FOR ODOR (olfactory evaluation)

Fig. 6

RECONSTITUTION OF SORGHUM GRITS WITH METHANOL EXRACTS FROM MILLET.


EXTRACTED SORGHUM GRITS
(100 gms)
+ MILLET METHANOL EXTRACT
+ SORGHUM METHANOL EXTRACT
AIR DRY
AIR DRY
WET (50 ml)
WATER
(50 ml) WET
WATER
TEST FOR ODOR
(olfactory evaluation)
TEST FOR ODOR
(olfactory evaluation)

ANALYTICAL PROCEDURES

HEXANAL DETERMINATION. The method of Fritsch and Gale (1977) was used to determine hexanal. An internal standard of 4-heptanone was prepared fresh by adding 25 ul of 4-heptanone to a 100 ml of distilled water.

A 15.0 gm sample of millet meal was transferred to an air tight bottle and two ml of internal standard added. Boiling distilled water was then added to make a total volume of 150 ml and the bottle immediately capped. The mixture was swirled for 45 seconds without interruption. Head space gas (5cc) was then withdrawn with an air tight syringe and injected into the gas chromatograph at a rate of 1 cc per second.

The gas liquid chromatograph used for hexanal determination was a Hewlett-Packard model with a flame ionization detector. Separation was on a 10' X 1/2" column of 10% silicone OV-101 on acid washed chromosorb W of 60-80 mesh. The column was maintained at 100° C, injection port at 200° C, and the detector block at 150° C.

To check the peak area response of hexanal relative to that of the internal standard heptanone, 5 ul each of 4-heptanone and hexanal were added to a 1000 ml of distilled water. A 10 ml sample was transferred to an air tight bottle with 15 gms of sucrose in it. Hexanal determination was carried as described above.

FATTY ACID ANALYSIS. Fatty acids were esterified and analyzed by a procedure developed by Dr. William Klopfenstein (Department of Biochemistry, Kansas State University, Manhattan, KS). A 20 gm sample of millet meal was extracted with petroleum ether for 12 hours using a Goldfish apparatus. The fat samples thus obtained were dissolved in a minimum volume of methanolic HCL or BCL_3 (Boron Trichloride). One ml of

benezene was added to this and incubated for 15 to 30 minutes at 110° C until the fat sample was totally dissolved. Hexane (1 ml) was added to the mixture and shaken vigorously. To this 1 to 2 ml of water was added. The sample was allowed to stand for a few seconds until a clear separation of the organic (hexane) layer on top and aqueous layer at the bottom was observed. The hexane layer was then pipetted off, and the sample re-extracted with another 1 ml of hexane. The two hexane extracts containing the esterified fatty acids were combined and washed with 0.5 ml of water. The water was pipetted off and the extracts dried with anhydrous sodium sulphate.

A 0.5 ul sample of the esterified fatty acids was analyzed with a Hewlett-Packard gas chromatograph equipped with a flame ionization detector. The column was 6' X 2 mm, packed with 7.5% DEGS on chromosorb G, of 100-200 mesh, (acid washed and DMCS treated). The column temperature was set a 150° C, injector port temperature at 220° C and detector block at 230° C.

Using the above method fat samples from freshly ground millet meal and also millet meal stored for ten days were analyzed for changes in fatty acids profiles.

PEROXIDE VALUES. The method was used as described by Tagaki et al (1978). Millet grits (50 gms) were weighed into a 100 ml volumetric flask and chloroform added to the mark. The contents were vigorously shaken and allowed to stand for five minutes after which the extract was filtered through Whatman ashless filter paper. A 5 ml aliquot of this sample was pipetted into a flask and 1 ml of 50% (W/V) aqueous KI solution, freshly prepared was added. The flask was stoppered and the mixture thoroughly shaken. After standing in darkness for five minutes,

the reaction mixture was diluted to the 25 ml mark of a volumetric flask with 2% aqueous cadmium acetate ($Cd(CH_3COO)$). The solution was shaken and placed in darkness until the two phases were clearly separated. The supernatent (aqueous) phase was decanted and its absorption measured spectrophotometrically against distilled water. The blank solution was prepared in a similar way but, without the millet grits. The spectrophotometer used was a Hitachi double beam recording spectrophotometer.

A caliberation curve was obtained as follows: 100 mg of $K_2 Cr_2 O_7$ was added to 100 ml of water. Six flasks each with 0.5 ml of HCL, 1 ml of KI and 10 ml of water were taken. The flasks were made to the mark by adding potassium dichromate and water in the ratio of 13.5:0, 12.0:1.5, 10.5:3, 9.0:4.5, 7.5:6.0, 6.0:7.5, respectively.

PEROXIDE VALUES OF MILLET LIPIDS. Pearl millet grits were extracted in a soxhlet for eight hours using 2000 ml of chloroform. The solvent was removed using a rotory evaporator. Varying amounts of fat samples (0.5, 1, 2, 4, 6, and 8 gms) were taken and iodine liberated was measured in each case following the procedure described above.

PEROXIDE VALUES OF GRAIN FRACTIONS. Millet grain was milled to give three fractions, bran germ and endosperm according to the method of Abdelrahman (1983). Each fraction (500 gms) was weighed into separate glass trays. Two hundred and fifty milliliters of water was added to each fraction and subsequently air dried. The peroxide values were determined using the procedure described previously.

PHENOLIC ACID DETERMINATION. The method followed was that of Hahn et al (1983), adapted for identification of phenolic acids in methanol extracts of millet grits. Methanol extract from both freshly ground as well as

from grits with off odor (treated grits) were analyzed. Phenolic acid standards, coumaric, caffeic, vanillic, ferulic, cinnamic, protocatechuic, and p-hydroxybenzoic acids were used. All the standards were obtained from Sigma Chemical Company. An equimolar mixture of all standards (1.0 X 10-4) in methanol was used as reference standard. The standard or sample (10 ul) was injected into Varian high pressure liquid chromatograph.

The column used was 30 cm X 1/4" R-Sil, C18 HL (10 u) particle diameter. Flow rate was 0.4 ml per minute. Gradient elution was performed using acetic acid: water (2:98) as solvent A and isopropanol: methanol (8:92) as solvent B. The eluent was programmed isocratically for 20 minutes at 5% solvent B, followed by a 13 min linear gradient to 15% solvent B. The mixture was pumped isocratically for 27 minutes at 15% solvent B. This was followed by a linear gradient for 20 minutes to 50% solvent B. The program was run for another 20 minutes isocratically at 50% solvent B and then linearly for 60 minutes to 100% solvent B.

SEP-PAK FRACTIONATION. Sep-Pak C18 perparatory columns were obtained from Waters Associates. The methanol extract (5 ml) from millet was passed through a 2 mm sep-pak column at a rate of 1 ml per second with pressure supplied by a gas syringe. The fraction eluted was called "eluent" and analyzed for phenolic acids. The material retained on the column was considered "retentate". It was washed off the column with large volumes of 100% methanol.

ACETAMIDE ANALYSIS. The procedure used was that described by Nakagawa et al (1971). The methanol extract from millet with off odor was analyzed for the presence of acetamide using a Perkin Elmer Model Sigma 300 with

flame ionization detector. The column was 4' X 1/8" aluminum tubing pecked with 100-200 mesh Chromosorb 101. Column temperature was 160° C, detector block was at 250 C and injector temperature was 250° C. Acetamide standard was obtained by dissolving 10 mg of pure acetamide in 20 ml of methanol. The standard or sample of 1 ul was injected for each chromatographic run.

UV SPECTRA

Ultra violet spectra were obtained in order to detect the presence of flavonoids. Apigenin (4', 5, 7-Trihydroxyflavone) was used as standard. Vitexin, isoVitexin, orientin, and iso Orientin are the 8- and 6- C-glucosyl derivatives of flavones apigenin and luteolin respectively. C-glycosylflavones have been synthesized in vitro from apigenin (Mabry et al, 1970). Apigenin is the only commercially available flavonoid related to vitexin, and was therefore, used in our study of flavonoid supplementation and UV scan.

Apigenin (1 mg) was dissolved in 5 ml of spectral grade methanol and the resulting solution was used as standard. The water soluble fraction of the methanol extract obtained from millet with off odor was used as sample. The water was removed by evaporation under nitrogen and the residue redissolved in 100% methanol. 100% methanol was used as a blank for each scan. The spectrophotometer (Varian model DMS 80) was operated at a scan rate of 50 nm per minute and standard and samples were scanned from 350 to 200 nm. Spectra were also obtained for sep-pak eluent and retentate of methanol extracts of millet.

SENSORY EVALUATION

A panel of ten members was selected for evaluation of off the odor generated in processed pearl millet during storage. The panel was

to test for the effect of day, time and day time interaction on the evaluation of each rater. Statistical tests were also done to find similarities and significant differences among the three different treatments.

RESULTS AND DISCUSSION

EFFECT OF STORAGE TIME

Samples from three crop years were initially investigated. This experiment was conducted to determine if there was any difference in odor production among millets harvested in different crop year; newly harvested (1982), that stored for one year (1981), and two years (1980). There was no particular trend in the effect of crop year on odor production among millets. Because there were no effect of storage time of the grain on odor generation, all three samples were used for the project. It was noted that at the relatively low moisture, it was difficult to generate strong odor in a short period of time (Table 1).

EFFECT OF MOISTURE LEVELS

Moisture content of the millet meal was found to be related both to the intensity and rate of odor generation. To test this, millet was tempered to 15.7%, 16.3% and 18.7% moisture. Millet of 14% moisture, from 1981 crop year was used as control. Control millet had very little odor even after 20 days of storage. The meal tempered to 18.5% moisture however, possessed a strong, objectionable odor within 30 hours of storage at room temperature. The three higher moisture samples had significant odor levels by the end of 72 hours. Thus, higher moisture levels were required to generate the off odor. These results agree with that of Lai and Varriano-Marston (1980b). This would suggest that the

Table 1

EFFECT OF STORAGE ON PROCESSED PEARL MILLET

SAMPLE	MOISTURE (%)	TIME (days) 3	5	7	10	20
1980						
A	10.4	F	F	S.R	S.R	S.R
B	10.3	F	F	F	F	S.R
C	10.3	F	F	F	R	S.R
1981						
D	13.1	F	S.R	S.R	R	R
E	13.7	F	S.R	S.R	S.R	R
F	14.0	S.R	S.R	S.R	S.R	R
1982						
G	7.8	F	F	F	S.R	S.R
H	7.4	F	F	S.R	S.R	S.R
I	7.2	F	F	S.R	R	S.R

F-- Fresh S.R-- Slightly rancid R-- Rancid

reaction(s) process resulting in the off odor formation in processed millet during storage might be enzymatic.

EFFECT OF SEQUENTIAL WETTING AND DRYING

In early studies, millet was ground to a fine meal in the Udy mill and stored in air tight jars prior to being tested for odor generation. It was found that this procedure was not effective in generating the odor. Certain millet samples could be stored for 20 days without generating appreciable levels of the above mentioned, mousy, acidic odor. During traditional processing of millet grain, water is added to soften the bran and thus, facilitate dehulling. The grits produced in this manner are subsequently dried and ground to flour. In an attempt to reproduce this process, a sequential wetting and frying of the grits was carried out and found to be effective in generating the off odor.

EFFECT ON TASTE

To determine whether or not a relationship existed between the generation of odor in the meal and a deterioration of taste of processed millet, a taste panel evaluation was carried out. The five member panel concluded unanimously that the gruel made from grits possessing the off odor had a bitter taste and was, therefore, inedible. The gruel made from freshly ground millet was starchy with a slight grainy aftertaste, but was not objectionable. This indicates that there is a positive correlation between odor production and deterioration of taste of millet meal.

HEXANAL DETERMINATION

Freshly ground millet meal, and millet meal stored for 10 days were analyzed for the presence of hexanal (fig. 8 and 9). No trace of hexanal was found in the meal even after 10 days of storage. Hexanal is a major

product of oxidative degradation of lipids. The fact that no hexanal was found indicates that there is no oxidative degradation of lipids even though odor was generated in the millet meal. These results agree with Kaced et al (1984).

FATTY ACID ANALYSIS

Oxidative rancidity is known to altar the fatty acid composition during storage. Fatty acid analysis of free lipids from fresh millet flour and fat from millet flour stored for periods up to ten days was carried out. Both methanolic Boron trichloride and methanolic HCL were used for esterification of the fatty acid standards and both were found to be effective (fig. 10 and 11). However, during preparation fat samples seemed to dissolve more rapidly in HCL than in BCL_3, therefore HCL was used in all the subsequent analyses. There were no significant differences in the composition of free fatty acids from millet meal with off odor (fig. 12) when compared to those of fresh millet meal (fig. 13). There was a slight increase in the amount of the C18 acids, oleic and linoleic, after 10 days of storage. However, this does not correlate well with the production of odor, which was detected after only three days of storage.

PEROXIDE VALUES

Peroxide values were obtained for the lipid extracted from millet meal with off odor (Table 2). In spite of the fact that the samples had objectionable odor their peroxide values were extremely low. Peroxide values were also determined on three principal milling fractions of the grain, bran, germ, and endosperm (Table 3). The peroxide values were highest for bran, followed by the endosperm and then germ. This however, is in reverse order for the odor generation. Peroxide values are

Fig. 7

GAS CHROMATOGRAM OF HEXANAL STANDARD. 1, n-Hexanal; 2, n-Heptanone.

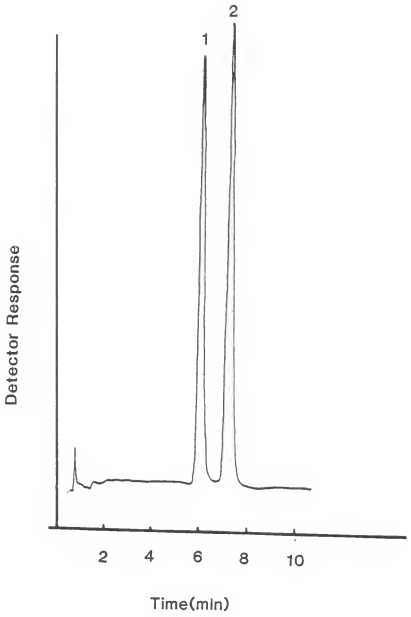

1
2
Detector Response
2
4
6
8
10
Time(min)

Fig. 8

HEXANAL DETERMINATION IN FRESHLY GROUND PEARL MILLET.

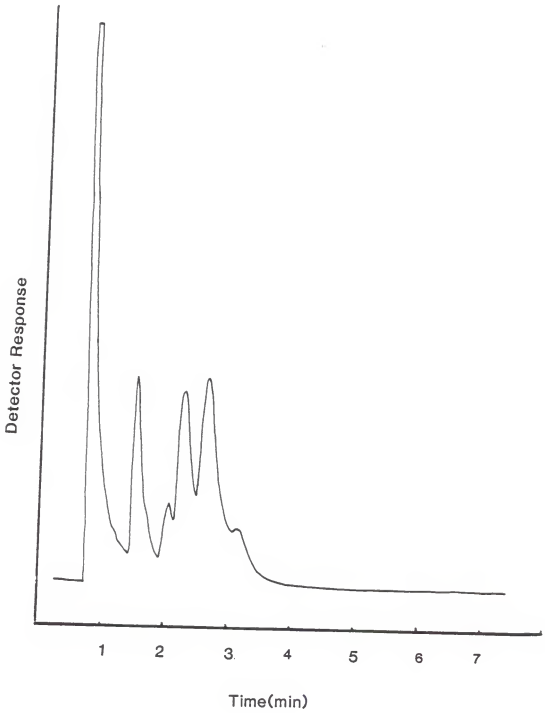
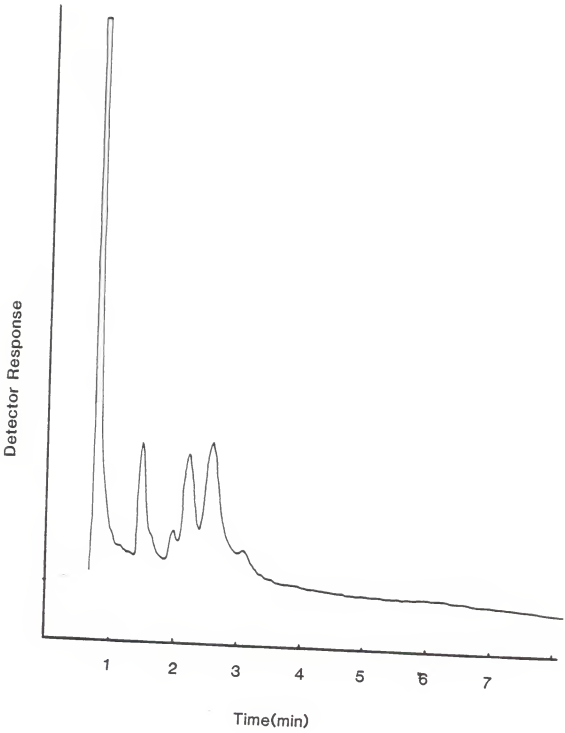

Detector Response
1
2
3
4
5
6
7
Time(min)

Fig. 9

HEXANAL DETERMINATION IN GROUND PEARL MILLET STORED FOR 10 DAYS.


Detector Response
1
2
3
4
5
6
7
Time(min)

indicative of oxidative changes in lipids. Our results show that the off odor we are finding may not be due to the decomposition of fat hydroperoxides.

FRACTIONATION AND RECONSTITUTION

We have concluded from the results of hexanal analysis, free fatty acid analysis and peroxide value determination, that the off odor generation was not related to oxidative rancidity of millet lipids. Therefore, identification of the compound(s) responsible for odor generation was undertaken. Millet grits were extracted with four solvents with widely differing polarities; petroleum ether, chloroform, methanol, and n-butanol. By testing the extracted grits as well as, grits reconstituted with their extracts (fig. 14), it was possible to evaluate the ability of each solvent to remove the odor generating compound(s) or the precursors. Results are presented in Table 4.

Although known to remove free lipids, petroleum ether extraction did not remove the odor causing compound(s). This reinforces our previous conclusions that oxidative rancidity of free lipids is not the cause of off odor generation. Chloroform extracted grits generated low but appreciable levels of odor whether butanol was a powerful solvent was difficult to evaluate in this study because of the strong residual solvent odor that remained even after prolonged drying. The strong odor masked any objectionable odor that may have been generated in the subsequent tests.

The fresh millet grits extracted with methanol removed the precursors of the odor causing substance while extraction of the wetted and dried grain was effective in removing the odor causing substance (fig. 15).

Table 4

EXTRACTION OF ODOR COMPONENTS AND RECONSTITUTION

SOLVENT	EXTRACTED GRITS	RECONSTITUTED GRITS
PETROLEUM ETHER	OFF ODOR	OFF ODOR
CHLOROFORM	OFF ODOR (less intense)	NO ODOR
METHANOL	NO OFF ODOR	OFF ODOR
n-BUTANOL	NO OFF ODOR	NO OFF ODOR

Fig. 10

FATTY ACID STANDARDS. Esterified with methanolic hydrochloric acid.

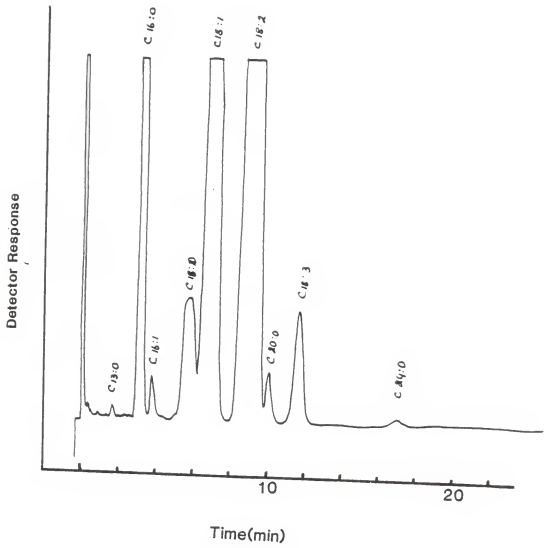

Detector Response
C 13:0
C 16:0
C 16:1
C 18:0
C 18:1
C 18:2
C 20:0
C 18:3
C 24:0
10
20
Time(min)

Fig. 11

FATTY ACID STANDARDS. Esterified with methanolic Boron Trichloride.

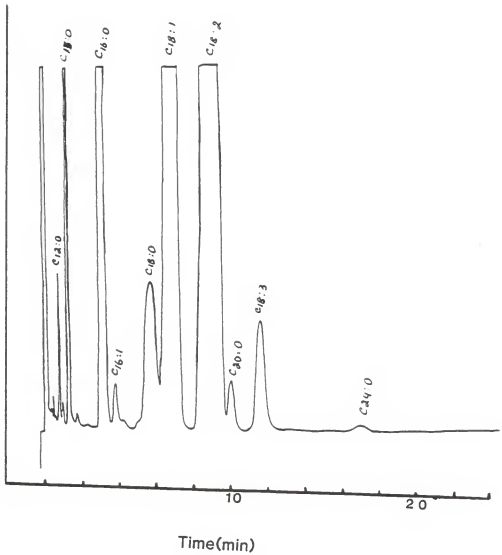

Detector Response
C12:0
C14:0
C16:0
C16:1
C18:0
C18:1
C18:2
C20:0
C18:3
C24:0
10
20
Time(min)

Fig. 12

GAS CHROMATOGRAM OF FREE, ESTERIFIED, FATTY ACIDS FROM PEARL MILLET STORED FOR 10 DAYS. 1, C13:0; 2, C16:0; 4, C18:0; 5, C18:1; 6, C18:2; 7, C20:0; 8, C18:3.

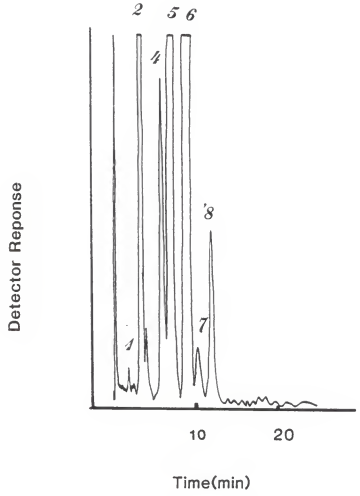

2
5
6
4
8
7
1
Detector Reponse
10
20
Time(min)

Fig. 13

GAS CHROMATOGRAM OF FREE, ESTERIFIED, FATTY ACIDS FROM FRESHLY GROUND PEARL MILLET. 1, C13:0; 2, C16:0; 3, C16:1; 4, C18:0; 5, C18:1; 6, C18:2; 7, C20:0; 8, C18:3.

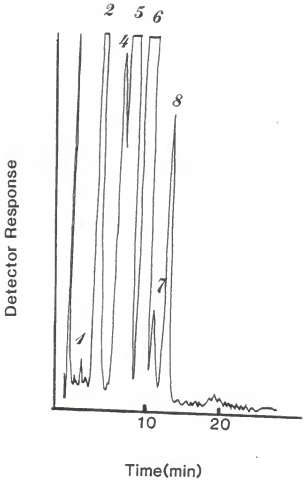

2
5
6
4
8
7
1
10
20
Detector Response
Time(min)

Table 2

PEROXIDE VALUE(POV) OF MILLET LIPIDS

LIPID (gms)	ABSORBANCE	POV(meq/kg of fat)
1	0.038	0.371
2	0.024	0.133
3	0.018	0.055
4	0.040	0.064
5	0.059	0.080
6	0.093	0.070

Table 3.

PEROXIDE VALUE(POV) OF MILLET FRACTIONS

FRACTION	ABSORBANCE	POV (meq/ kg of fat)
BLANK	0.004	0.0116
WHOLE	0.004	0.0116
BRAN	0.016	0.0206
ENDOSPERM	0.004	0.0116
GERM	0.005	0.0123

Fig. 14

RECONSTITUTION OF MILLET GRITS WITH THE METHAOL EXTRACT.

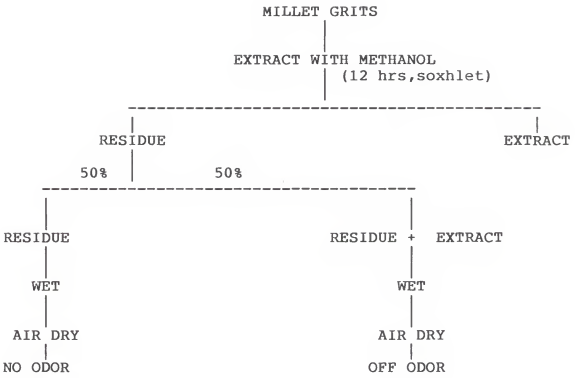
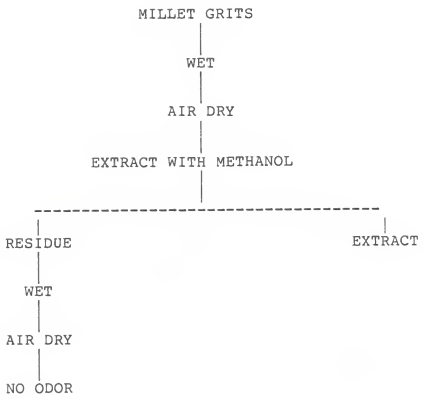

MILLET GRITS
EXTRACT WITH METHANOL
(12 hrs,soxhlet)
RESIDUE
EXTRACT
50%
50%
RESIDUE
RESIDUE + EXTRACT
WET
WET
AIR DRY
AIR DRY
NO ODOR
OFF ODOR

Fig. 15

EXTRACTION OF MILLET GRITS WITH METHANOL AFTER THE OFF ODOR IS PRODUCED.


MILLET GRITS
WET
AIR DRY
EXTRACT WITH METHANOL
RESIDUE
EXTRACT
WET
AIR DRY
NO ODOR

FRACTIONATION OF THE METHANOL EXTRACT

The methanol extract removed the putative precursor of the odor compounds. It was further divided into "water solubles" and "methanol solubles" fractions. The methanol extract was dried and water was added to it. The solubles were filtered out giving a water solubles extract. The residual material was redissolved in methanol and used as methanol solubles (see materials and methods for details of the procedure). Both fractions had the pale yellow color of the original methanol extract. The two fractions were tested for their ability to produce odor. The "water soluble" fraction was functional whereas, the "methanol soluble" fraction did not produce any off odor. In most plant extracts it is reported that the bulk of low molecular weight phenolic material is present in water soluble fraction as glycosides.

PHENOLIC ACID DETERMINATION

Methanol extracts of millets were found to contain caffeic acid, p-coumaric acid, ferulic acid and cinnamic acid. This conclusion was based on comparison of retention times during a gradient elution, to the retention times of the standard mixture (fig. 16). The analysis procedure was a gradient elution, from 50% solvent B (methanol: isopropanol: 92:8) to 100% solvent B for another 100 min. A number of unidentified, high molecular weight compounds were eluted in this region (fig. 17). These results indicated that the methanol extract contained a large quantity of compounds besides phenolic acids. Therefore, it was thought essential to fractionate the extract.

SEP-PAK FRACTIONATION

Hahn et al (1983) reported that the methanol extracts of sorghum containing phenolic acids were passed through sep-pak columns to clean up

Fig. 16

STANDARD PHENOLIC ACIDS: 1)Gallic acid (2)protocatechuic acid (3)p-Hydroxy benzoic acid (4)Vanillic acid (5 & 6)Caffiec (7 & 8)p-Coumaric acid (9 & 10)Ferulic acid (11)Cinnamic acid

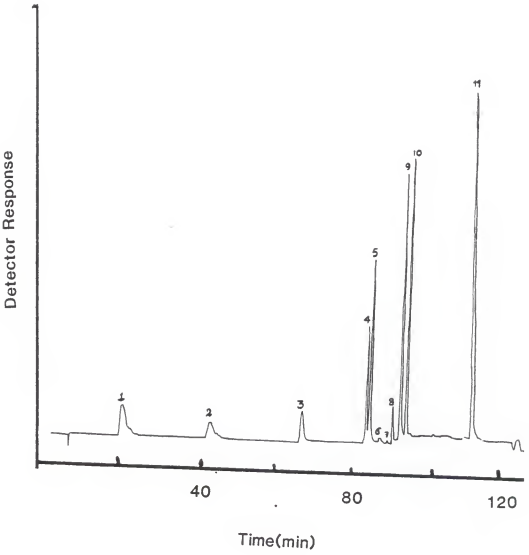
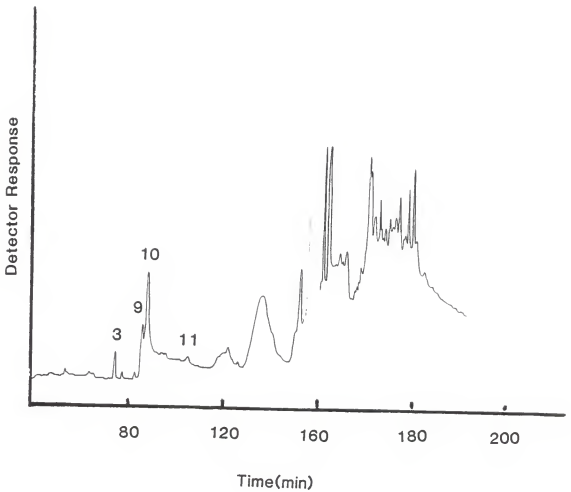

Detector Response
1
2
3
4
5
6
7
8
9
10
11
40
80
120
Time(min)

Fig. 17

PHENOLIC ACIDS IN THE METHANOL EXTRACT OF PEARL MILLET. 3, P-Hydroxy benzoic acid; 9,10, ferulic acid; 11, cinnamic acid.


Detector Response
10
9
3
11
80
120
160
180
200
Time(min)

the extract. They found that high molecular weight polyphenols in the sample were retained as a yellow-brown band in the top few millimeters of the column. Based on this the methanol extract from millet was passed through sep-pak columns and separated into two fractions. The fraction termed "eluent", was found to contain the phenolic acids (fig. 18). The "retentate" fraction was found to contain the unidentified, high molecular weight substances (fig. 19).

Each fraction was reconstituted separately with the methanol extracted grits, wetted, dried and tested for odor. The eluent fraction did not produce an off odor with the millet grits. The retentate fraction was "active" in that it produced a definite off odor with the grits. This indicates that the unidentified, high molecular weight substances found in millet extract are responsible for producing the mousy, acidic odor in the processed millet.

INTERCHANGE STUDIES

This test was undertaken to verify whether the off odor was specific to millet (procedure is described in Materials and Methods section). The methanol extracts of sorghum and millet were interchanged. Extracted sorghum grits reconstituted with methanol extract from millet when wetted and dried generated the off odor. Conversely extracted millet grits, reconstituted with methanol extract from sorghum did not produce any off odor even after wetting and drying. This shows that the methanol extract of millet has a specific compound(s) which is active in generation of the off odor.

ACETAMIDE ANALYSIS

The off odor in millet can be characterized as typically mousy and acidic. It was suggested (Whitaker, 1984, oral communication) that

Fig. 18

PHENOLIC ACIDS IN SEP-PAK RETENTATE OF METHANOL EXTRACT FROM FRESHLY GROUND PEARL MILLET. 2, Protocatechuic acid; 4,5, ferulic acid.

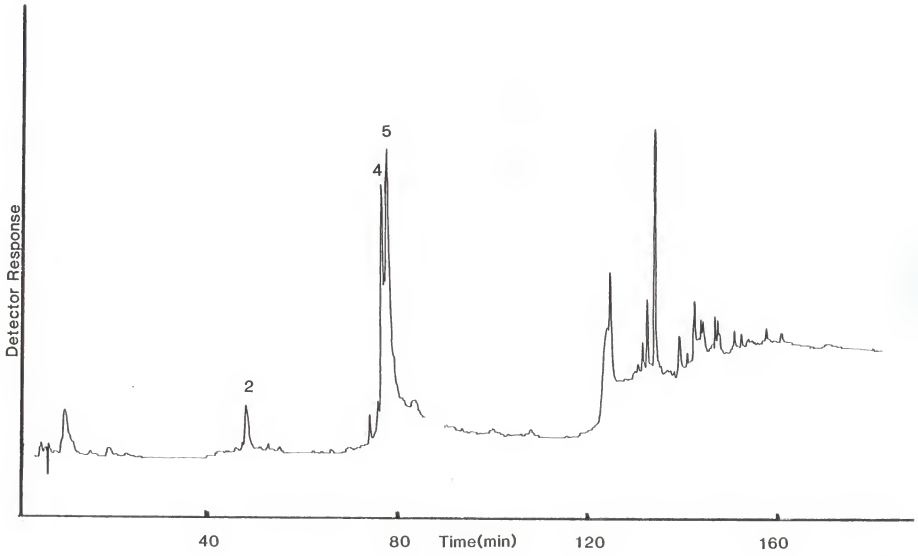
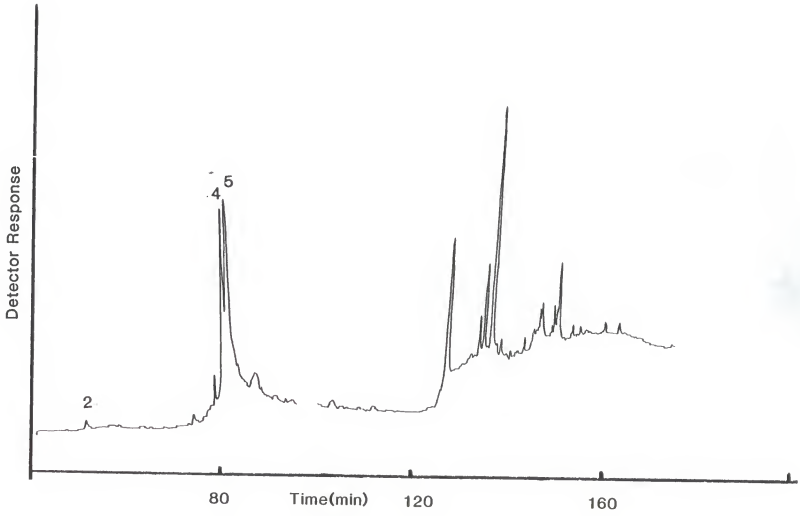

Detector Response
5
4
2
40
80
Time(min)
120
160

Fig. 19

PHENOLIC ACIDS IN SEP-PAK ELUENT OF METHANOL EXTRACT FROM PEARL MILLET. 2, Protocatechuic acid; 4, 5, ferulic acid.


Detector Response
2
4
5
80
Time(min)
120
160

acetamide which is odorless when pure, but frequently has a mousy odor may be the source of the odor. Acetamide is soluble in both water and methanol, but more soluble in water. Methanol extract of millet was therefore, analyzed for the presence of acetamide. The method of analysis for acetamide by Nakagawaer (1971) was found to be simple and efficient. The standard eluted a sharp peak with a retention time of 4.2 minutes (fig. 20). In standard dilution assays, acetamide could be detected at concentrations as low as 0.001 mg/ml of methanol. Methanol extracts from millet grits possessing the characteristic off odor were analyzed, but no traces of acetamide were found. We concluded that the off odor was not a result of presence of acetamide in processed pearl millet.

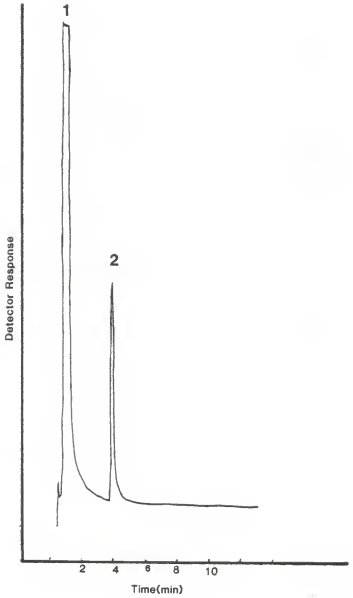
UV SPECTRA

Hahn et al (1983) used sep-pak columns to remove polyphenols from the extract they used for detection of phenolic acids as a purification step. Because the sep-pak retentate which contained the high molecular weight substances effectively generated the odor in millet grits, detection of polyphenols was considered. In particular flavonoids which are the most important and widely occurring class of polyphenols. Ultra-violet spectrascopy is an important tool used in identification of flavonoids. UV spectra of flavones and flavonols in methanol exhibit two major peaks, band I has max values at 300-380 nm, and bank II has in the region of 240-280 nm. Band I is considered to be associated with absorption due to the B-ring cinnamoyl system and band II to the A-ring benzoyl system.

Both the extracts, from fresh grain and grain with off odor had similar spectra. The UV absorption maxima were in three regions, 312 nm,

Fig. 20

GAS CHROMATOGRAM OF ACETAMIDE STANDARD. 1, Solvent (CH_3OH); 2, Acetamide.


1
2
Detector Response
2
4
6
8
10
Time(min)

287 nm, and 270 nm. Apigenin standards were found to have a max at 312 nm, 270 nm, and 210 nm, (fig. 21). "Water solubles" from the methanol extract of millet had the same regions of UV absorbency as apigenin standard (fig. 22). This suggests that the flavonoids found in millet may be a part of the enzymtic process resulting in formation of off odor in pearl millet.

SENSORY EVALUATION

Results of the statistical analyses show that there was no significant effect of day and time on the evaluation of each panelist (Table 5). There is also no significant effect of day and time interaction on treatment. This indicates that the procedure for generating off odor is both reliable and reproducible. Lease Significant Difference (LSD), Duncan's Multiple Range Test and Bonferroni (Dunn) t-test, were performed on the data to compare the three treatments for the presence and intensity of odor (Table 7 and 8).

The results were similar for the three tests. The millet grits which were sequentially wetted and dried had the highest intensity of off odor. The other two treatments, millet grits reconstituted with methanol extract and grits supplemented with apigenin had slightly lower intensity of off odor.

These results indicate that the flavonoids present in pearl millet (Reichert, 1979) are the active components resulting in off odor generation in processed millet during storage. The intensity of off odor was same for methanol reconstituted and apigenin supplemented millet grits. One possibility is due to partial denaturation of the enzyme(s) involved in the odor generating reaction during extraction with methanol.

Fig. 21

UV SPECTRA OF APIGENIN STANDARD. ABSORPTION MAXIMA 312 NM, 270 NM, 210 NM.

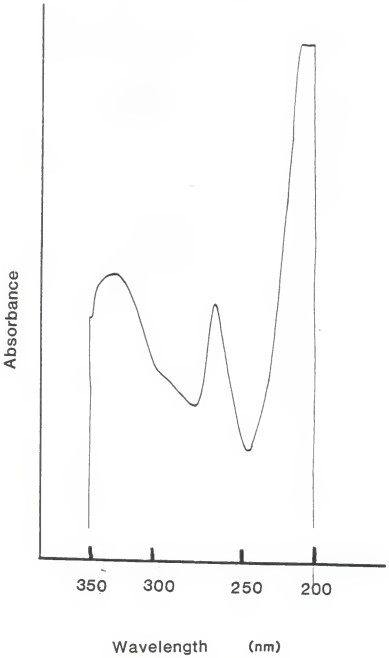

Absorbance
350
300
250
200
Wavelength (nm)

Fig. 22

UV SPECTRA OF WATER SOLUBLES. ABSORPTION MAXIMA 312 NM, 270 NM, 210 NM.

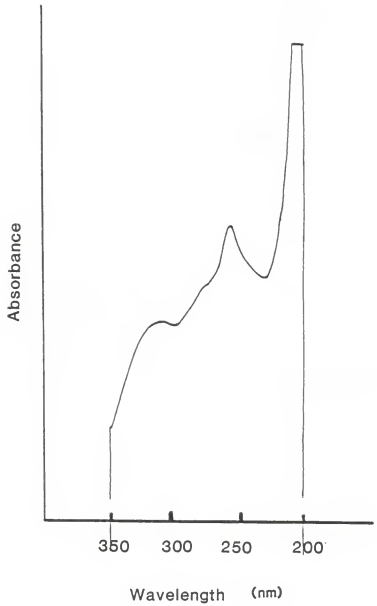

Absorbance
350
300
250
200
Wavelength (nm)

Table 5

ANALYSIS OF VARIANCE OF DATA FROM SENSORY EVALUATION

SOURCE	DF	SUM OF SQUARE	MEAN SQUARE	F VALUE	PR > F
MODEL	7	102.93	14.704	8.65	0.0001
ERROR	32	54.39	1.699		
CORRECTED	39				

Dependent variable : score

R-Square = 0.654

Table 6

ANALYSIS OF VARIANCE OF DATA FROM SENSORY EVALUATION

SOURCE	DF	SUM OF SQUARES	F VALUE	PR > F
TREATMENT	3	99.57	19.53	0.0001
RATER	3	3.36	0.49	0.7399

Table 7

T- TEST (LSD)

GROUPING	MEAN	N	TREATMENT
A	5.395	10	SEQUENTIAL WETTING & DRYING
B	3.775	10	METHANOL EXTRACT RECONSTITUTED
B	3.025	10	APIGENIN SUPPLEMENTED
C	1.005	10	FRESHLY GROUND

VARIABLE : SCORE

Means with the same letter are NOT significantly different.

Table 8

BONFERRONI (DUNN) T TESTS

GROUPING	MEAN	N	TREATMENT
A	5.395	10	SEQUENTIALLY WETTED & DRIED
B,A	3.775	10	METHANOL EXTRACT RECONSTITUTED
B	3.025	10	APIGENIN SUPPLEMENTED
C	1.005	10	FRESHLY GROUND

VARIABLE : SCORE

Means with the same letters are NOT significantly different.

CONCLUSIONS

No traces of hexanal were found in millet stored for up to ten days, although the initiation of off odor production was found as early as 24 hours. The peroxide values of the millet meal were extremely low in samples stored for varying length of time. There were no significant changes in the fatty acid profile of the millet lipid stored for ten days. These results indicate that oxidative rancidity of millet lipid is not responsible for the off odor found in the meal, during storage.

Moisture content of the processed millet was found to be an important factor in odor generation. The time taken for the initiation and the intensity of off odor having a direct positive correlation to the amount of moisture in the millet. It might indicate that off odor generation is an enzymatic process.

There was a deterioration in taste of millet meal that had the off odor. The meal was found to taste bitter and was ranked as inedible by a taste panel as compared to the freshly ground meal, which was starchy with a slight grainy aftertaste.

Petroleum ether and chloroform were not effective in extracting precursors of the odor component(s). Methanol was found to successfully extract the odor component(s), without altering the functional properties significantly. This indicates once again that oxidative rancidity of free lipids does not contribute to the deterioration of processed millet during storage. Petroleum ether pre-extraction was found to give a cleaner extract, more suitable for further analysis. Reconstitution of the methanol extract of millet with residual, methanol extracted sorghum was found to generate the typical acidic, mousy odor in the sorghum but not vice versa.

Fractionation of the methanol extract on sep-pak columns yielded a fraction called "retentate", which was effective in generating the off odor in residual millet grits.

The methanol extract of millet also contained water soluble material. A UV scan of the water soluble fraction had absorption maxima at 312 nm, 270 nm and 212 nm. This was similar to that of apigenin standard.

Sensory evaluation data indicated that reconstitution of methanol extract of the millet with extracted grits produced the same intensity of odor as grits supplemented with apigenin. This shows that the off odor in millet is because of the C-glucosylflavonoid compounds millet contains.

The intensity of odor was slightly higher in the control millet grits which were sequentially wetted and dried. This may indicate that there might be partial denaturation of the enzyme(s) involved in the reaction that results in off odor generation, during extraction with methanol.

Further research is necessary to determine the mechanism of the process resulting in off odor generation. Recent studies indicate that flavonoids in plants are catabolized into phenolic acids and eventually volatile products by a lipoxygenase type of enzyme (Harborne et al, 1975). Since flavonoid content of the grain was found to decrease markedly, but not totally, after decortication (Reichert, 1979) development of a better milling process might not improve the storage quality of processed millet.

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THE IDENTIFICATION OF THE PRECURSOR OF OFF ODOR CAUSING COMPOUNDS, PRODUCED DURING STORAGE OF GROUND PEARL MILLET(*Pennisetum americanum* [L] Leeke)

by

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AN ABSRACT OF A MASTER'S THESIS

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ABSTRACT

Home processing of pearl millet includes adding moisture to the grain in order to soften the bran for decortication during milling. The higher moisture content was found to be an important factor contributing to the deterioration of ground millet. The deterioration results in the production of an acidic, mousy odor.

A taste panel found that the millet meal was off odor had a bitter, unacceptable taste unlike, the freshly ground meal. Absence of hexanal, low peroxide values, and no significant changes in free fatty acid composition, indicated that there was no oxidative rancidity of millet lipids during the production of the off odor.

The precursor(s) and product(s) of the reaction resulting in off odor production were effectively extracted with methanol. The methanol extract was fractionated using sep-pak cartridges. The fraction retained on the sep-pak column was found to contain the active material.

UV spectra of flavonoid apigenin was similar to that of water soluble fraction of the methanol extract from millet. Supplementation of the methanol extracted millet grits with apigenin was compared to reconstitution of millet grits with methanol extract. Results of sensory evaluation showed that the two treatments had the same intensity of mousy, acidic off odor. It was therefore, concluded that flavonoids present in millet are responsible for off odor production.